Development of biomarkers utilizing variable lymphocyte receptors (VLRS) of inshore hagfish (Eptatretus burgeri) against avian influenza virus H9N2

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Hagfish, along with lampreys, are jawless vertebrates (agnathans) which do not have essential adaptive components, such as T-(TCRs) and B-(BCRs or Ig) cell receptors and MHC molecules, which are possessed by their jawed (gnathostomes) counterparts. They have lymphocytes similar to T and B cells that are referred to as variable lymphocyte receptors (VLRs). VLRs are proteins made up of leucine-rich repeats (LRRs) that are assembled into functional receptors through somatic diversification of germ-line VLR (gVLR) gene/s in agnathans. Lampreys and hagfish have two VLRs , VLR-A and VLR-B, which are known to be equivalent to TCRs and BCRs in vertebrates, respectively. The VLR gene can generate a diverse repertoire of these cell surface receptors comparable to the predicted diversity of mammalian antibody repertoire. This suggests that VLRs serve as jawless fish equivalents of the anticipatory antigen receptors of jawed vertebrates and are sufficient to recognize a wide range of antigenic determinants. The unique phylogenetic position of VLRs in the evolution of adaptive immunity provides many potential advantages in VLR research. In particular, VLRs function as the immunoglobulin (Ig)-based system of jawed vertebrates, which, together with their relatively small size and high stability, enhances the potential of VLRs for biomarkers beyond the higher vertebrates-derived antibodies. In this study, we tried to isolate and express monoclonal VLR-B proteins specific for low-pathogenic avian influenza virus (LPAIV) subtype H9N2 hemagglutinin (H9) protein by using a monoclonal antibody against VLR-B. We already reported from a previous study that hagfish immunized with LPAIV H9 showed robust plasma VLRB responses, demonstrating adaptive immune responses against the antigens. Here, we constructed large VLRB libraries from antigen-stimulated hagfish cDNA in a yeast surface-display system, with the VLR C-terminal fused to the yeast Aga surface anchor. This high-throughput screening system enables us to isolate monoclonal VLRs and explore the role of VLR in hagfish immunity by screening large libraries for specific antigen-binding VLR clones. The ability of hagfish immunity to produce a repertoire of mature VLRs in response to specific antigens makes it a good candidate for antibody therapies and may be beneficial in treating significant diseases.

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